Claims

1. A method for producing a nutrient medium for growing mammalian or insect cells in culture whereby for at least one of H, C or N, substantially all atoms in substrates that are used by the cells for synthesis of biomolecules in the nutrient medium are isotopically labelled, whereby the method comprising the steps of:

(a) growing an organism on a mineral medium which supports growth of the organism, whereby in the medium substantially all of the assimilable atoms, for at least one of H, C or N, are isotopically labelled, to produce labelled biomass;

(b) autolysing the biomass of an organism grown as in (a) to produce an autolysate; and,

(c) composing the nutrient medium by combining the autolysate as obtained in (b) with further components necessary for growth of the mammalian or insect cells.

2. A method according to claim 1, wherein the organism is a fungus, yeast or algae.

3. A method according to claim 2, wherein the organism is an organism that belongs to a genus selected from *Saccharomyces, Pichia, Hansenula, Kluyveromyces, Candida, Brettanomyces, Debaryomyces, Tolrulopsis, Yarrowia, Galdieria, Cyanidium, Porphyridium, Cystoclonium, Audouinella,* and *Cyanidioschyzon.*

4. A method according to any one of claims 1 – 3, wherein the method further comprises the steps of:

(a) growing an organism on a mineral medium which supports growth of the organism, whereby in the medium substantially all of the assimilable atoms, for at least one of H, C or N, are isotopically labelled, to produce labelled biomass;

(b) extracting biomass of an organism with an organic solvent to produce an extract comprising lipids, whereby the organism is grown as in (a) or is grown as in (a) on a medium without isotopic substitution;

(c) hydrolysing biomass of an organism grown as in (a) at a non-alkaline pH to produce a hydrolysate comprising amino acids;

(d) composing the nutrient medium by combining the autolysate as obtained in any one of claims 1 - 3 with amino acids as obtained in (c) and adding further components necessary for growth of the mammalian or insect cells.

5. A methods according to claim 4, wherein in step (d) the nutrient medium is composed by combining the autolysate obtained in any one of claims 1 – 3 with the amino acids obtained in (c) and the lipids obtained in (b) and adding further components necessary for growth of the mammalian or insect cells.

6. A method according to any one of claim 1 - 5, whereby the nutrient medium is composed of autolysate, lipids and amino acids obtained from at least two different organisms.

7. A method according to any one of claims 1 - 6, whereby, prior to hydrolysis in (c), lipids and pigments are extracted from the biomass using an organic solvent.

8. A method according to any one of claims 1 - 7, whereby the organism from which the lipids are extracted, belongs to a genus selected from the group consisting of *Rhodophyta*, *Cyanidiophyceae*, *Chlorophyta*, *Cyanophyta*, *Diatoms*, *Phaeophyceae*, *Dinoflagelate*, *Dinophyta* and *Galdieria*.

9. A method according to any one of claims 1 - 8, whereby the organism from which the hydrolysate comprising amino acids is produced, is an organism selected from the group consisting of algae, fungi, yeasts and methylotrophic bacteria.

10. A method according to claim 9, whereby the organism belongs to a genus selected from the group consisting of *Pichia*, *Saccharomyces*, *Hansenula*, *Cyanidium*, *Galdieria*, *Porphyridium*, *Spirulina*, and *Methylobacillus*.

11. A method according to any one of claims 1 - 10, whereby the further components necessary for growth of the mammalian or insect cells comprise one or more of:
(a) one or more of glucose, fructose, and sucrose;

(b) one or more Krebs-cycles intermediates selected from the group consisting of citrate, succinate, fumarate, maleic acid, oxalate and malate;
(c) pyruvate; and,
(d) one or more vitamins selected from the group consisting of thiamin, riboflavin, niacin, vitamin B6, folic acid, vitamin B12, biotin, pantothenic acid, choline, para-aminobenzoic acid and alpha-tocopherol.

12. A method according to any one of claims 1 - 10, whereby substantially all atoms in substrates that are used by the mammalian or insect cells for synthesis of biomolecules in the nutrient medium are isotopically labelled with an isotope selected from ^{15}N; ^{13}C; ^{2}H; ^{15}N and ^{13}C; ^{15}N and ^{2}H; ^{13}C and ^{2}H; or ^{15}N, ^{13}C and ^{2}H.

13. A method for producing a biomolecule, whereby substantially all atoms in the biomolecule are isotopically labelled, the method comprising the steps of:
(a) growing a culture of mammalian or insect cells capable of producing the biomolecule under conditions conducive to the production of the biomolecule, in a nutrient medium produced in a method according to any one of claims 1 - 12; and
(b) recovery of the biomolecule.

14. A method according to claim 13, wherein the biomolecule is a soluble protein or a membrane protein.

15. A method according to claim 14, wherein the mammalian or insect cells capable of producing the protein comprise an expression vector comprising a nucleotide sequence coding for the protein.

16. A method according to claims 14 or 15, wherein the protein is a mammalian protein.

17. A method for obtaining structural information on a biomolecule, the method comprising the steps of:
(a) producing a biomolecule, whereby substantially all atoms in the biomolecule are isotopically labelled, in a method according to any one of claims 13 - 15;

(b) optionally, purifying the biomolecule;

(c) subjecting the biomolecule to spectroscopic analysis to obtain information about its structure.

18. A method according to claim 17, wherein the spectroscopic analysis comprises NMR spectroscopy.

19. A method according to claims 17 or 18, wherein the structural information on a biomolecule is information about the three-dimensional structure of the biomolecule.

20. A method according to any one of claims 17 - 19, wherein the biomolecule is a protein complexed to a second biomolecule.

21. A method according to claim 20, wherein the second biomolecule is produced in a method according to any one of claims 1 - 10 and whereby 20 - 100% of the hydrogen atoms in the second biomolecule are uniformly substituted with the isotope ^{2}H.

22. A method according to claim 21, wherein the second biomolecule is a protein.

23. A nutrient medium for the production of an isotopically labelled biomolecule from mammalian or insect cells, the medium supporting growth of a mammalian or insect cell culture under condition conducive to the production of the biomolecule, the medium comprising:

(a) a mixture of inorganic salts;

(b) a source of amino acids;

(c) a carbohydrate energy source;

(d) a source of lipids;

(e) optionally, a protective agent;

(f) optionally, vitamins and/or organic compounds;

(g) optionally, organic acids; and,

(h) optionally, trace elements;

whereby substantially all atoms in (a), (b), and (c), and, optionally in (d), (e), (f), (g) and (h) are isotopically labelled for at least one of H, C or N or whereby 20 - 100% of the hydrogen atoms in (a), (b), and (c), and, optionally in (d), (e), (f), (g) and (h) are uniformly substituted with the isotope ^{2}H.

24. A nutrient medium according to claim 23, whereby the source of amino acids comprises an hydrolysate comprising amino acids that is produced from yeast biomass, whereby the hydrolysis of the biomass comprises autohydrolysis.

25. A nutrient medium according to claims 23 or 24, whereby the source of lipids comprises fatty acids, steroids, and lipid soluble vitamins.

26. A nutrient medium according to any one of claims 23 - 25, whereby the carbohydrate energy source is one or more of glucose, fructose, and sucrose; the organic acids are one or more of pyruvate and the Krebs-cycles intermediates selected from the group consisting of citrate, succinate, fumarate, maleic acid, oxalate and malate; the vitamins are one or more vitamins selected from the group consisting of thiamin, riboflavin, niacin, vitamin B6, folic acid, vitamin B12, biotin, pantothenic acid, choline, para-aminobenzoic acid and alpha-tocopherol.

27. A nutrient medium according to any one of claims 23 - 26, whereby substantially all atoms in (a), (b), and (c), and, optionally in (d), (e), (f), (g) and (h) are isotopically labelled with an isotope selected from ^{15}N; ^{13}C; ^{2}H; ^{15}N and ^{13}C; ^{15}N and ^{2}H; ^{13}C and ^{2}H; or ^{15}N, ^{13}C and ^{2}H.

28. A mammalian membrane protein whereby substantially all atoms in the protein are isotopically labelled with an isotope selected from ^{15}N, ^{13}C, ^{2}H, ^{15}N and ^{13}C, ^{15}N and ^{2}H, ^{13}C and ^{2}H, or ^{15}N, ^{13}C and ^{2}H.

29. A mammalian membrane protein whereby 20 - 100% of the hydrogen atoms in the protein are uniformly substituted with the isotope ^{2}H.

30. A mammalian membrane protein according to claims 28 or 29, whereby the protein is a human protein.